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Effect of hereditary obesity on renal expressions of NO synthase, caveolin-1, AKt, guanylate cyclase, and calmodulin

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Background. Obesity has emerged as a major cause of diabetes, cardiovascular disease, and renal insufficiency worldwide. Obese Zucker rats exhibit hyperphagia, obesity, insulin resistance, hyperlipidemia, and glomerulosclerosis and are frequently used as a model to study hereditary form of metabolic syndrome. Nitric oxide plays a major role in preservation of renal function and structure. The present study was designed to test the hypothesis that renal disease in this model may be associated with down-regulation of endothelial (eNOS) and neuromal NO synthases (nNOS) in the kidney. The study further sought to explore expressions of caveolin-1, phospho AKt, and calmodulin, which regulate activities of constituitive NOS isoforms, as well as soluble guanylate cyclase (sGC), which is involved in NO signaling.

Methods. Twenty-two-week-old male obese and lean Zucker rats were studied. Body weight, serum lipids, urine albumin excretion, and renal tissue abundance of the above proteins were determined.

Results. Serum glucose and arterial pressure were unchanged, whereas urinary NO metabolite (NO_χ) excretion and renal tissue nitrotyrosine abundance were markedly reduced (denoting depressed NO production) in the obese versus lean Zucker rats. This was accompanied by significant glomerulosclerosis, tubulointerstitial damage, renal immune cell infiltration, marked down-regulations of renal tissue eNOS and nNOS, mild reduction of caveolin-1, and unchanged calmodulin, phospho-AKt, and sGC.

Conclusion. Hereditary obesity can result in down-regulations of kidney eNOS and nNOS, marked reduction of NO production, and glomerulosclerosis prior to the onset of frank diabetes and hypertension.

Obesity has emerged as a major cause of diabetes, renal disease, and cardiovascular complications worldwide. Pathologic obesity can cause glomerular hypertrophy, mesangial expansion, focal segmental glomerulosclerosis, and glomerular proteinuria in humans and experimental animals [1–4]. In addition, obesity is associated with an increased risk of type 2 diabetes, which is the leading cause of cardiovascular disease and chronic renal failure [5–8]. Various animal models have been employed to investigate the pathogenesis of nephropathy associated with obesity and type 2 diabetes [1, 3, 9, 10]. One such model is the obese Zucker rat (fa/fa-rat) [1, 3, 10], in which an autosomal-recessive mutation of the fa-gene, encoding the leptin receptor, results in hyperphagia, obesity, hyperlipidemia, peripheral insulin resistance, hyperinsulinemia, and impaired glucose tolerance [11, 12]. With increasing age, these rats develop proteinuria and focal segmental glomerulosclerosis [1, 3, 10, 13].

Nitric oxide (NO) plays a major role in regulation of arterial pressure as well as renal and cardiovascular function and structure. Numerous studies have explored NO production and NO synthase activity in the vascular tissues of the Zucker obese rats [14–22]. However, the available data on expressions of NO synthase (NOS) isoforms and proteins involved in regulation of NOS activity and NO signaling in the kidney of Zucker rats are limited. Therefore, this study was conducted to explore the expressions of constitutive isoforms of NOS, as well as key proteins involved in regulation of their enzymatic activities (phospho AKt, calmodulin, and caveolin-1) and NO signaling (soluble guanylate cyclase) in the kidneys of obese and lean Zucker rats.

METHODS

Animals

Twenty-two-week-old male Zucker obese and lean rats were used (Harlan Sprague, Inc., Indianapolis, IN, USA). The animals were housed in a climate-controlled and light-regulated room (12 hour light/12 hour dark cycle). Food and water were provided ad libitum. Body weight and tail arterial pressure (tail plethysmography) were measured and a 24-hour urine collection was obtained using metabolic cages. Urine specimens were stored at

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−20°C until assayed. The animals were then anesthetized (nembutal, 50 mg/kg, IP) and euthanized by exsanguinations using cardiac puncture between the hours of 10 and 12 AM. The left kidney was immediately removed, cleaned, snap frozen in liquid nitrogen, and stored at −70°C until processed. The remaining kidney was fixed in 10% formalin and used for histologic and immunohistologic examination.

Tissue preparation

Kidney cortex was separated and homogenized in 10 mmol/L HEPES buffer, pH 7.4, containing 320 nmol/L sucrose, 1 mmol/L EDTA, 1 mmol/L DTT, 10 mg/mL leupeptin, 2 mg/mL aprotinin, and 1 μmol/L phenylmethylsulfonyl fluoride (PMSF) at 0 to 4°C. Homogenates were centrifuged at 12,000*g* for 5 minutes at 4°C to remove tissue debris and nuclear fragments. The supernatant was used to perform the Western analyses. Total protein concentration was determined with the use of a Bio-Rad kit (Bio-Rad Laboratories, Hercules, CA, USA).

Western blot analyses

Protein abundance of endothelial NOS (eNOS), neuronal NOS (nNOS), caveolin-1, phospho AKt, calmodulin, and soluble guanylate cyclase (sGC) were measured by Western blot analysis as described in our earlier studies [23, 24]. Antibody against B subunit of sGC was purchased from Calbiochem (San Diego, CA, USA). Anti-caveolin-1 antibody was purchased from Affinity Bioreagents, Inc. (Golden, CO, USA). Antibodies to phospho AKt, eNOS, and nNOS were purchased from BD Biosciences (San Diego, CA, USA). On each occasion, the Ponceau S staining was used to verify the uniformity of protein load and transfer efficiency across the test samples. Experiments failing this test were discarded.

Measurements of urine total NO_2^- plus NO_3^- (NO_χ)

The NO_χ concentration in the urine samples was determined as described earlier [25] using the purge system of a Sievers Instruments, Inc. (Boulder, CO, USA). In a typical assay, 10 μL of the sample was injected into the purge vessel, and all samples were run in triplicates. The amount of NO_χ in the sample was determined by interpolation of the result in the standard curve.

Histology and immunohistology

Light microscopy studies were performed in formalin-fixed paraffin embedded kidney sections stained with periodic acid–Schiff (PAS) and hematoxylin and eosin (H&E) stainings. All histologic and immunohistologic studies were done blindly without previous knowledge of the experimental groups. Severity of glomerulosclerosis was evaluated by determining the number of glomeruli showing sclerosis and the area within the glomerulus showing sclerosis. At least 32 glomeruli were examined in each biopsy (range 32–66). As described in previous communications [26–28], the glomeruli were graded from 0 to +4 (Grade 0 = normal, grade 1 = <25% involvement of the glomerular tuft, grade 2 = 25–50% involvement of the glomerular tuft, grade 3 = 50–75% and grade 4 = sclerosis occupying >75% of the glomerular tuft). The total glomerulosclerosis score was calculated as follows: [(1× *N* glomeruli with +1 score) + (2 × *N* glomeruli with +2 score) + (3 × *N* glomeruli with +3 score) + (4 × *N* glomeruli with +4 score)]/total number of glomeruli examined.

Tubulointerstitial damage (infiltration, fibrosis, tubular dilatation, or atrophy) was evaluated using a damage score as described previously [26, 28, 29]. The grading was done according to the extent of the damaged tubulointerstitial area in the renal cortex (0 = normal, grade +1 = <10%, grade +2 = 10–25%, grade +3 = 25–50%, grade +4 = 50–75%, grade +5 = 75–100%). The extent of tubulointerstitial damage was determined in successive fields examined in the entire cortical and juxtamedullary areas, suitable for evaluation, in each specimen. Damaged areas were defined visually in each ×200 field and quantified as a percent of the total area under examination using computer assisted image analysis (Optimas, Media Cybernetics, Silver Spring, MD, USA).

Avidin-biotin-peroxidase methodology was used to identify lymphocytes (CD5 positive cells) and macrophages (ED1 positive cells) as described in previous communications [30]. Cellular infiltration was evaluated separately in the glomeruli and in tubulointerstitial areas and expressed as positive cells per glomerular cross section (gcs) or positive cells per mm^2, respectively.

Antisera

Monoclonal antibodies were used to identify lymphocytes (anti-CD5 clone MRCOX19; Biosource, Camarillo, CA, USA) and macrophages (anti ED1; Harlan Bioproducts, Indianapolis, IN, USA). Secondary rat anti-mouse and donkey anti-rabbit antibodies with minimal cross-reactivity to rat serum proteins were obtained from Accurate Chemical and Scientific Co. (Westbury, NY, USA).

Miscellaneous tests

Urinary albumin and creatinine concentrations were measured using Nephrat kit and Creatinine Companion kit purchased from Exocell, Inc. (Philadelphia, PA, USA). Plasma glucose was measured using Beckman glucose analyzer (Beckman Coulter, Fullerton, CA, USA). Serum cholesterol, triglyceride, and creatinine were determined by AnTech Diagnostics (Irvine, CA, USA).

Data presentation and analysis

Data are presented as mean ± SEM. Student *t* test was used in statistical evaluation of the data. P values ≤ 0.05 were considered significant.

Table 1. Body weight, blood pressure, plasma lipid, albumin, creatinine, and random plasma glucose levels

	Lean	Obese
Body weight *g*	423 ± 8	688 ± 11[c]
Serum cholesterol *mg/dL*	79 ± 6	164 ± 13[c]
Serum triglyceride *mg/dL*	87 ± 18	549 ± 106[b]
Urinary albumin excretion *mg/day*	6 ± 3	15 ± 2[a]
Serum creatinine *mg/dL*	0.30 ± 0.04	0.3 ± 0.1[a]
Blood pressure *mm Hg*	145 ± 10	131 ± 8
Random plasma glucose *mg/dL*	218 ± 14	277 ± 54

[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$. $N = 6$ in each group

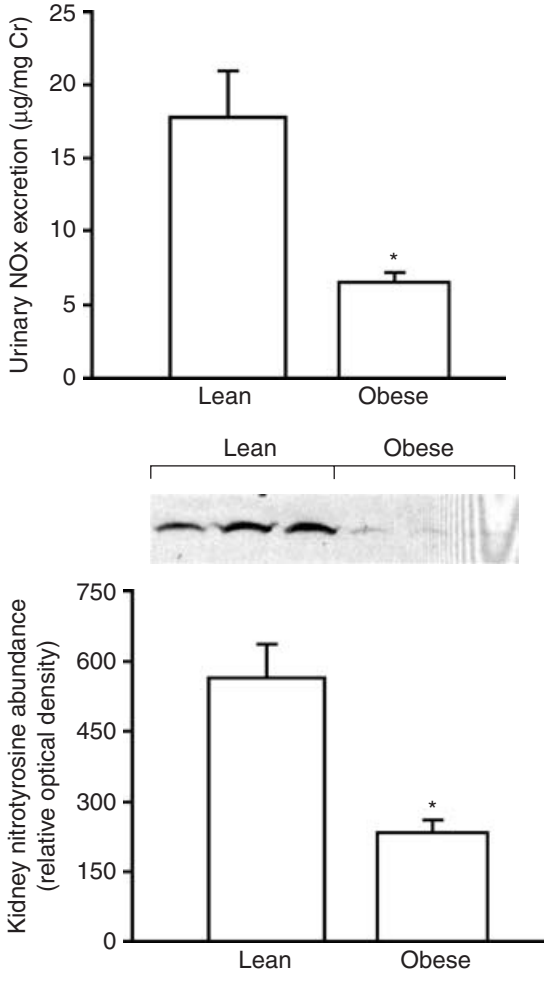

Fig. 1. Urinary NO_χ excretion (upper panel) and representative Western blots and group data depicting nitrotyrosine abundance in the kidney cortex (lower panel) of obese and lean Zucker rats. $N = 6$ in each group, $^*P < 0.05$.

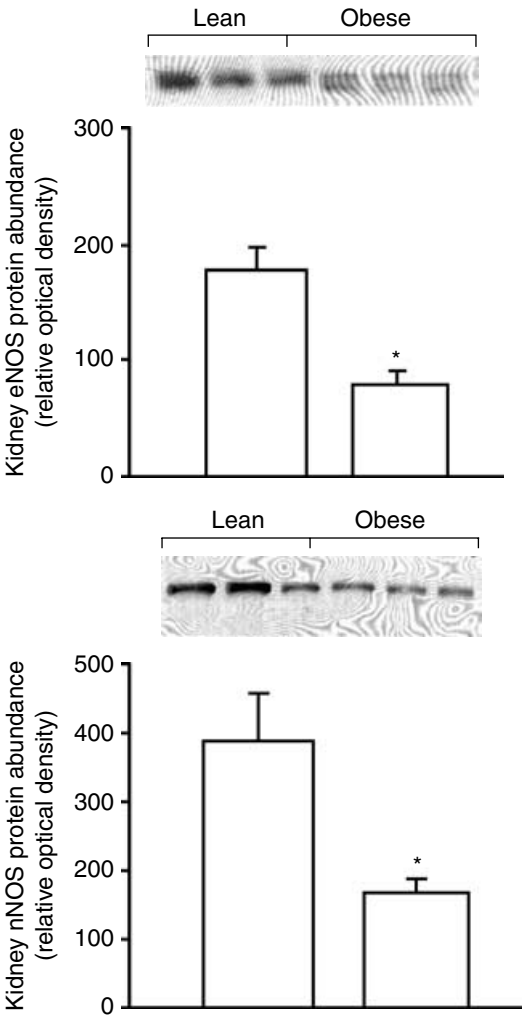

Fig. 2. Representative Western blots and group data depicting eNOS and nNOS protein abundance in the kidney cortex of the obese and lean Zucker rats. $N = 6$ in each group, $^*P < 0.05$.

RESULTS

General data

Data are summarized in Table 1. As expected, body weight in the obese Zucker rats was significantly greater than in the lean Zucker rats. Obese Zucker rats exhibited elevated urinary albumin excretion, confirming earlier studies [1, 10, 13, 29]. Likewise, serum cholesterol and triglyceride concentrations were significantly elevated in the in the obese Zucker rats as compared to the lean controls. However, arterial pressure and serum glucose concentrations were not significantly different among the two groups.

NOS, nitrotyrosine, and urinary NO_χ data

Data are depicted in Figures 1 and 2. The eNOS abundance in the renal cortex was significantly lower in the obese Zucker rats compared to that found in the lean Zucker rats. Similarly, nNOS protein abundance in the renal cortex was significantly lower in the obese Zucker rats than in the lean Zucker rats. The reduction of renal tissue eNOS and nNOS protein abundance in the obese Zucker rats was accompanied by a significant reduction of tissue nitrotyrosine abundance and urinary NO_χ excretion, pointing to reduced NO production in these animals.

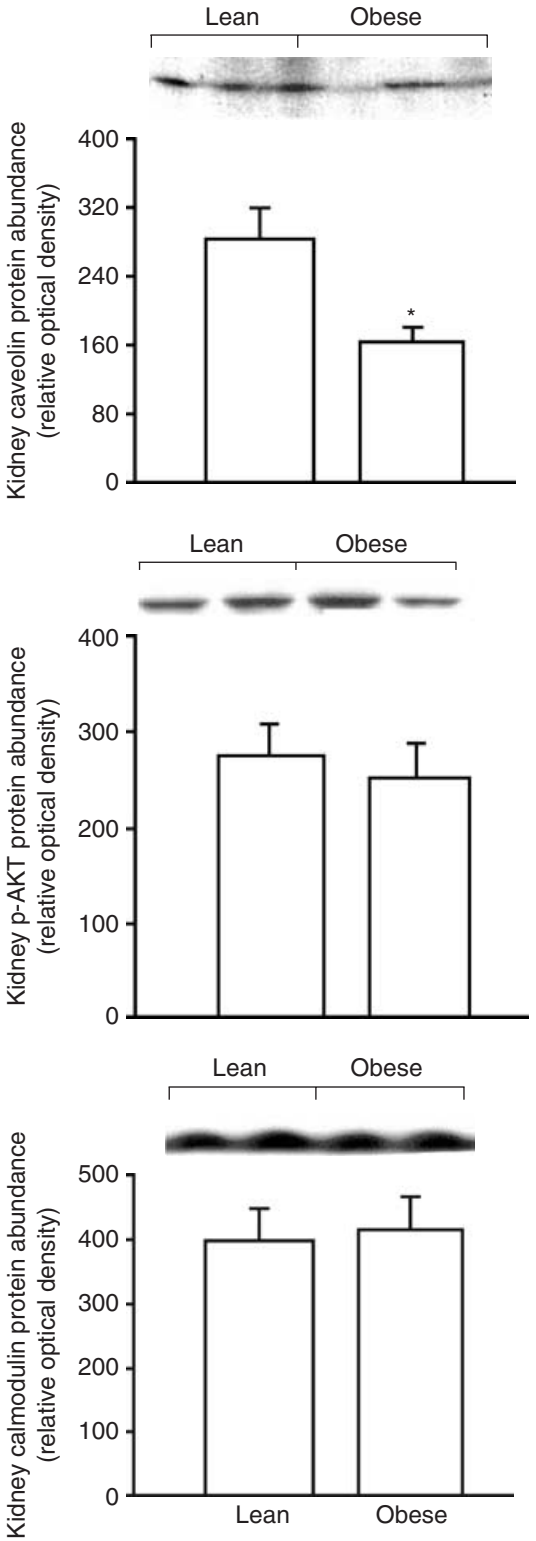

Fig. 3. Representative Western blots and group data depicting protein abundance of caveolin-1, phospho-AKt, and calmodulin in the kidney cortex of obese and lean Zucker rats. $N = 6$ in each group, $^{*}P < 0.05$.

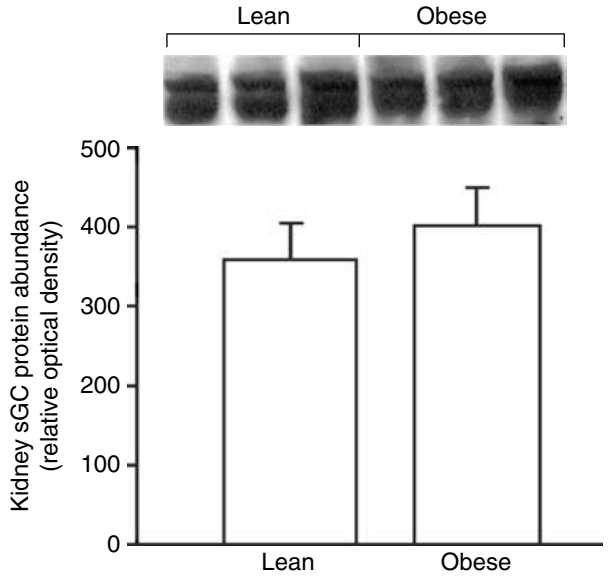

Fig. 4. Representative Western blot and group data depicting soluble guanylate cyclase (sGC) protein abundance in the kidney cortex of the obese and lean Zucker rats. $N = 6$ in each group, no differences found.

Tissue, caveolin-1, calmodulin, phospho-AKt, and sGC data

Data are shown in Figures 3 and 4. There was no significant difference in calmodulin protein abundance in the kidney cortex (Fig. 6) among the obese and lean Zucker rats. Likewise, no significant difference was found in either phospho-AKt or sGC protein abundance in the kidney cortex among the obese and lean Zucker rats. In contrast, caveolin-1 abundance was significantly lower in the obese than in the lean Zucker rats.

Histology and immunohistology

Data are illustrated in Figure 5A to F. Glomerular and tubulointerstitial damage scores were significantly higher in the obese Zucker rats than in the lean Zucker rats, which had essentially normal histology. Glomerular macrophage and lymphocyte infiltration (CD5-positive cells) were significantly increased in the glomeruli and tubulointerstitial regions in the obese Zucker rats.

DISCUSSION

Compared to the lean Zucker rats, obese Zucker rats employed in the present study showed significant increases in body weight, serum cholesterol, and triglyceride levels at 22 weeks of age. However, at this stage, arterial blood pressure and serum glucose concentration in the obese Zucker rats were not significantly different from the corresponding values found in the lean Zucker rats.

Despite absence of frank diabetes and hypertension, our obese Zucker rats exhibited significant albuminuria,

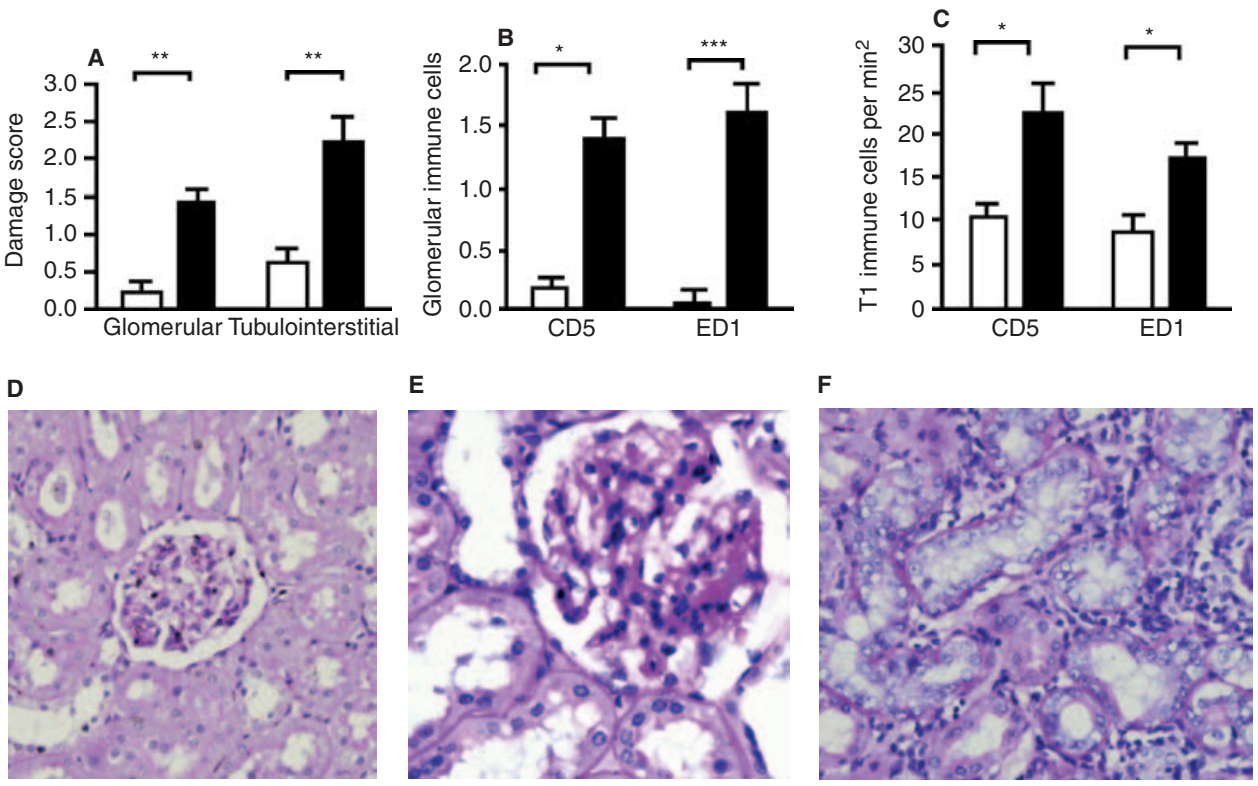

Fig. 5. Glomerular and tubulointerstitial damage scores (*A*), and glomerular (*B*) and tubulointerstitial (*C*) infiltration of lymphocytes (CD5 positive cells) and macrophages (ED1 positive cells). Values in obese Zucker rats (closed columns) are contrasted with those in the lean Zucker rats (open columns). $^{*}P < 0.05$, $^{**}P < 0.05$, $^{***}P < 0.001$. Microphotographs that illustrate an essentially normal appearance in the lean Zucker rats (*D*) and various abnormalities in obese Zucker rats including focal glomerulosclerosis (arrow) in a glomerulus surrounded by some dilated tubules (*E*), and tubulointerstitial injury (*F*) characterized by focal cellular infiltration (arrow heads) and tubules showing intraluminal lipid droplets (asterisks), as well as intracellular vacuolization (arrows) (all microphotographs are PAS staining, original magnification ×400). Also known are the macrophage (ED-1) infiltration scores in the kidney cortex of the obese and lean Zucker rats.

confirming the occurrence of kidney disease. This observation is consistent with the results of the earlier studies in this model [1, 3, 10, 13, 29]. The presence of albuminuria was accompanied by significant glomerulosclerosis in the 22-week-old obese Zucker rats.

Previous studies have demonstrated that monocyte/macrophage influx precedes the development of glomerulosclerosis in the obese Zucker rat [31]. The findings of the present study confirm the occurrence of glomerulosclerosis and tubulointerstitial damage, as well as glomerular and tubulointerstitial infiltration of immune cells in the obese Zucker rats. Since macrophages/monocytes play a role in the pathogenesis of focal and diffuse glomerulosclerosis in chronic nephropathies of various etiologies [32], it is likely that they are also involved in the pathogenesis of nephropathy in the obese Zucker rat.

NO plays a major role in the regulation of vascular resistance and regional and systemic hemodynamics. NO generated by different NOS isoforms in the kidney contributes to the regulation of renal vascular resistance, renal blood flow, glomerular filtration rate, and renin secretion [33–37]. In addition, NO promotes sodium excretion directly by suppressing tubular sodium reabsorption and indirectly by modulating renal medullary blood flow [38, 39]. Besides its critical role in regulation of renal and vascular functions, NO serves as a potent inhibitor of platelet activation, cell adhesion, proliferation, and matrix accumulation. Consequently, a reduction in NO bioavailability can contribute to inflammation, cell proliferation, and matrix expansion.

The presence of glomerulosclerosis and albuminuria in the 22-week-old obese Zucker rats was accompanied by significant reductions of eNOS and nNOS abundance in the kidney cortex. The observed down-regulation of NOS isotypes in the renal cortex was accompanied by a significant reduction of urinary excretion of NO metabolites (NO_χ) and tissue nitrotyrosine abundance. Together, the reduction in urinary excretion of NO_χ and renal tissue abundance of nitrotyrosine point to diminished NO production in the obese Zucker rats and, as such, provides functional evidence for the observed down-regulation of NOS isoforms in this model.

Activation of eNOS and nNOS is dependent on calcium-mediated binding of calmodulin to the inactive enzyme. Moreover, eNOS activity is inhibited by its

binding to caveolin-1 and markedly amplified by phospho AKt-catalyzed phosphorylation of the enzyme [40]. In an attempt to further explore the mechanism(s) responsible for diminished NO production in obese Zucker rats, we determined the abundance of these important NOS-regulatory proteins as well. The study revealed a significant reduction of caveolin-1 and no significant change in the abundance of either calmodulin or phospho-AKt in the obese Zucker rats, thus excluding dysregulation of these proteins as a possible cause of diminished NO production in these animals.

Activation of soluble guanylate cyclase by NO results in generation of cGMP, which mediates many of the biological actions of NO. We therefore sought to explore the possible effect of obesity on the abundance of this key enzyme in the kidneys of the study animals. The results showed no significant difference in the abundance of soluble guanylate cyclase between the obese and lean Zucker rats.

Recent studies have revealed significant down-regulation of kidney NOS isotypes and diminished NO availability in the genetically normal rats with diet-induced obesity and metabolic syndrome without frank diabetes [41]. It thus appears that both genetic and diet-induced obesity can diminish NO availability that is accompanied by, and partly due to, down-regulation of NOS isotypes in the kidney. It is of note that proteinuria in rats with puromycin-induced glomerular proteinuria results in down-regulation of nNOS expression in the kidney [42]. Thus, the obesity-associated proteinuria may play a part in the development of nNOS deficiency in this model. In addition, diabetes and simulated hyperglycemia have been shown to down-regulate eNOS in vivo and in vitro, respectively [43, 44]. However, animals employed in the present study did not have frank diabetes. Finally, hyperlipidemia is known to cause endothelial dysfunction and limit NO availability. Therefore, presence of severe hyperlipidemia in the obese Zucker rats may have contributed to the observed down-regulations of NOS isotypes.

CONCLUSION

Taken together, these observations suggest that obesity and hyperlipidemia in the absence of frank diabetes and hypertension can lead to down-regulation of renal tissue eNOS and nNOS, as well as significant glomerulosclerosis and albuminuria in this model.

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REFERENCES

1. Kasiske BL, Cleary MP, O'Donnell MP, *et al*: Effects of genetic obesity on renal structure and function in the Zucker rat. *J Lab Clin Med* 106:598–604, 1985
2. Kasiske BL, Crosson JT: Renal disease in patients with massive obesity. *Arch Intern Med* 146:1105–1109, 1986
3. Kasiske BL, O'Donnell MP, Keane WF: The Zucker rat model of obesity, insulin resistance, hyperlipidemia, and renal injury. *Hypertension* 19:I110–I115, 1992
4. Praga M, Hernandez E, Andres A, *et al*: Effects of body-weight loss and captopril treatment on proteinuria associated with obesity. *Nephron* 70:35–41, 1995
5. Ascherio A, Rimm EB, Giovannucci EL, *et al*: A prospective study of nutritional factors and hypertension among US men. *Circulation* 86:1475–1484, 1992
6. Hall JE: Renal and cardiovascular mechanisms of hypertension in obesity. *Hypertension* 23:381–394, 1994
7. Hsueh WA, Buchanan TA: Obesity and hypertension. *Endocr Hypertension* 23:405–427, 1994
8. White SL, Cass A, Atkins RC, *et al*: Chronic kidney disease in the general population. *Adv Chronic Kidney Dis* 12:5–13, 2005
9. Velasquez MT, Kimmel PL, Michaelis OE: Animal models of spontaneous diabetic kidney disease. *FASEB J* 4:2850–2859, 1990
10. Zucker LM: Hereditary obesity in the rat associated with hyperglycemia. *Ann NY Acad Sci* 131:447–458, 1965
11. Ionescu E, Sauter JF, Jeanrenaud B: Abnormal oral glucose tolerance in genetically obese (fa/fa) rats. *Am J Physiol* 248:E500–E506, 1985
12. Phillips MS, Liu Q, Hammond HA, *et al*: Leptin receptor missense mutation in the fatty Zucker rat. *Nat Genet* 13:18–19, 1996
13. Gades MD, Goor HV, Kaysen GA, *et al*: Brief periods of hyperphagia cause renal injury in the obese Zucker rat. *Kidney Int* 56:1779–1787, 1999
14. Fruheck G: Pivotal role of nitric oxide in the control of blood pressure after leptin administration. *Diabetes* 46:903–908, 1999
15. Giordano A, Tonello C, Bulbarelli A, *et al*: Evidence for a functional nitric oxide synthase system in brown adipocyte nucleus. *FEBS Letters* 514:135–140, 2002
16. Karagiannis J, Reid JJ, Darby I, *et al*: Impaired nitric oxide function in the basilar artery of the obese Zucker rats. *J Cardiovasc Pharmacol* 42:497–505, 2003
17. Kawaguchi M, Koshimura K, Sohmiya M, *et al*: Effect of insulin on nitric oxide synthase-like immunostaining of arteries in various organs in Zucker diabetic fatty rats. *Eur J Endocri* 145:343–349, 2001
18. Kuboki K, Jiang ZY, Takahara N, *et al*: Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo: A specific vascular action of insulin. *Circulation* 101:676–681, 2000
19. Laight DW, Desai KM, Anggard EE, *et al*: Endothelial dysfunction accompanies a pro-oxidant, pro-diabetic challenge in the insulin resistant, obese Zucker rat in vivo. *Eur J Pharmacol* 402:95–99, 2000
20. Morley JE, Mattammal MB: Nitric oxide synthase levels in obese Zucker rats. *Neuroscience Letters* 209:137–139, 1996
21. Schwaniger R, Sun H, Mayhan WG: Impaired nitric oxide synthase-dependent dilation of cerebral arterioles in type II diabetic rats. *Life Sci* 73:3415–3425, 2003
22. Stepp DW, Frisbee JC: Augmented adrenergic vasoconstriction in hypertensive diabetic obese Zucker rats. *Am J Physiol Heart Circ Physiol* 282:H816–820, 2002
23. Sindhu RK, Ehdaei A, Vaziri ND, *et al*: Effect of chronic renal failure on caveolin-1, soluble guanylate cyclase and AKt protein expression. *Biochem, Biophys Acta* 1690:231–237, 2004
24. Vaziri ND, Ding Y, Sangha DS, *et al*: Upregulation of NOS by simulated microgravity, potential cause of orthostatic intolerance. *J Appl Physiol* 89:338–344, 2000
25. Vaziri ND, Ni Z, Wang XQ, *et al*: Downregulation of nitric oxide synthase in chronic renal insufficiency: Role of excess PTH. *Am J Physiol, Renal Physiol* 43:F642–F649, 1998
26. Alvarez V, Quiroz Y, Nava M, *et al*: Overload proteinuria is followed by salt-sensitive hypertension caused by renal infiltration of immune cells. *Am J Physiol (Renal Physiol)* 283:F1132–1141, 2002
27. Rodriguez-Iturbe B, Quiroz Y, Ferrebuz A, *et al*: Evolution of renal interstitial inflammation and NF-kB activation in spontaneously hypertensive rats. *Am J Nephrol* 24:587–594, 2004

28. Rodriguez-Iturbe B, Quiroz Y, Nava M, *et al*: Reduction of renal immune cell infiltration results in blood pressure control in genetically hypertensive rats. *Am J Physiol (Renal Physiol)* 282:F191–F201, 2002
29. Coimbra TM, Janssen U, Grone HJ, *et al*: Early events leading to renal injury in obese Zucker (fatty) rats with type II diabetes. *Kidney Int* 37:167–182, 2000
30. Parra G, Hernandez S, Moreno P, *et al*: Participation of the tubulointerstitium in acute immune-complex nephritis: Interstitial antigen accumulation, cellular infiltrate and MCH class I expression. *Clin Exp Immunol* 133:44–49, 2003
31. Lavaud S, Michel O, Sassy-Prigent C, *et al*: Early influx of glomerular macrophages precedes glomerulosclerosis in the obese Zucker rat model. *J Am Soc Nephrol* 7:2604–2615, 1996
32. Rodríguez-Iturbe B, Pons H, Herrera-Acosta J, *et al*: The role of immunocompetent cells in non-immune renal diseases. *Kidney Int* 59:1626–1640, 2001
33. Mattson DL, Bellehumeur TD: Neural nitric oxide synthase in renal medulla and blood pressure regulation. *Hypertension* 28:297–303, 1996
34. Romero JC, Lahera V, Salom MG, *et al*: Role of the endothelium-dependent relaxing factor nitric oxide on renal function. *J Am Soc Nephrol* 2:1371–1387, 1992
35. Singh I, Grams M, Wang WH, *et al*: Coordinate regulation of renal expression of nitric oxide synthase, rennin, and angiotensinogen mRNA by dietary salt. *Am J Physiol* 270:F1027–1037, 1996
36. Tolins JP, Shultz PJ: Endogenous nitric oxide synthesis determines sensitivity to the pressure effect of salt. *Kidney Int* 46:230–236, 1994
37. Ying WZ, Sanders PW: Dietary salt enhances glomerular endothelial nitric oxide synthase through TGF B1. *Am J Physiol* 275:F18–24, 1998
38. Gabbai FB, Blantz RC: Role of nitric oxide in renal hemodynamics. *Semin Nephrol* 19:242–250, 1999
39. Mattson DL, Lu S, Nakanishi K, *et al*: Effect of chronic renal medullary nitric oxide inhibition on blood pressure. *Am J Physiol* 266:H1918–926, 1994
40. Michel T: Targeting and translocation of endothelial nitric oxide synthase. *Braz J Med Biol Res* 32:1361–1366, 1999
41. Roberts CK, Barnard JR, Jurezak M, *et al*: A high-fat refined carbohydrate diet induces endothelial dysfunction, oxidant antioxidant imbalance and depressed NO synthase expression. *J Appl Physiol* 98:203–210, 2005
42. Ni Z Vaziri ND: Downregulation of nitric oxide synthase in nephrotic syndrome: Role of proteinuria. *Biochem Biophys ACTA* 1638:129–137, 2003
43. Ding Y, Vaziri ND, Coulson R, *et al*: Effect of simulated hyperglycemia, insulin, and glucagons on endothelial nitric oxide synthase expression. *Am J Physiol Endocrinol Metab* 279:E11–17, 2000
44. Koo JR, Vaziri ND: Effects of diabetes, insulin and antioxidants on NO synthase abundance and NO interaction with reactive oxygen species. *Kidney Int* 63:195–201, 2003